American Journal of Hematology 58:267–272 (1998)

Thrombopoietin Level Is Inversely Related to Blast Count, not Platelet Number, in Down Syndrome Neonates With Transient Myeloproliferative Disorder

Motoki Bonno,[1]* Eiichi Azuma,[1,2] Hajime Kawasaki,[1] Xao-Li Zhang,[1] Yoshihiro Komada,[1] Masahiro Hirayama,[1] Masamune Higashigawa,[3] Masakazu Umemoto,[1] Tadashi Koike,[4] Takashi Kato,[5] Tomoyuki Tahara,[5] Hiroshi Miyazaki,[5] and Minoru Sakurai[1]

[1]Department of Pediatrics, Mie University School of Medicine, Tsu, Mie, Japan
[2]Department of Clinical Immunology, Mie University School of Medicine, Tsu, Mie, Japan
[3]Department of Perinatal Care Unit, Mie University School of Medicine, Tsu, Mie, Japan
[4]Bone Marrow Transplantation Unit, Niigata University School of Medicine, Niigata, Japan
[5]Pharmaceutical Research Laboratory, Kirin Brewery Co. Ltd., Takasaki, Gunma, Japan

Transient myeloproliferative disorder (TMD) in neonates with Down syndrome is characterized by increased megakaryoblastic cells in the peripheral blood. Despite their spontaneous regression in weeks, prognosis is not always favorable because of fatal hepatic fibrosis. In this study, blood thrombopoietin (TPO) levels were measured by ELISA in six TMD patients and the expression of c-Mpl, a ligand for TPO, was examined on the blast cells from four patients by flow cytometer. At the onset, TPO level was undetectable in one patient and significantly lower in five patients than six neonatal controls (mean 0.52 fmol/ml, range 0.30–0.93 vs. 3.70, 1.38–8.33, $P < 0.001$), although platelet counts were similar (mean 321 × 10^9/l, range 42–1,040 vs. 253 × 10^9/l, 124–381). Two patients died of hepatic failure. TPO levels were measured in five patients after regression of the blast cells. With regression of blast cells, TPO levels were remarkably increased in four survived patients. In one patient with hepatic failure, TPO level was poorly elevated and relatively lower compared to the others. TPO levels were inversely correlated with blast numbers ($r = -0.85$, $P < 0.001$), but not with platelet counts ($r = 0.426$). Blast cells from four patients were all positive for c-Mpl. Our findings suggest that megakaryocyte mass is a major regulator of TPO levels and hepatic failure may affect the TPO level because liver is a major source of TPO production. Am. J. Hematol. 58:267–272, 1998.

Key words: transient myeloproliferative disorder (TMD); thrombopoietin; c-Mpl; Down syndrome; megakaryopoiesis

INTRODUCTION

Children with Down syndrome have an increased risk of leukemia, and especially megakaryoblastic leukemia is closely associated with Down syndrome [1,2]. In addition, it is known that hematological abnormalities indistinguishable from acute megakaryoblastic leukemia occur frequently in neonates with Down syndrome. This disorder, called transient myeloproliferative disorder (TMD), is characterized by an increased number of abnormal blasts in the peripheral blood and their spontaneous regression in several weeks without chemotherapy [3]. Despite the fact that the blasts regress spontaneously, prognosis of TMD is not always favorable because of fatal hepatic fibrosis [4]. Pathogenesis of TMD is still unknown; however, trisomy 21 is thought to play an important role in abnormal megakaryopoiesis [5–7].

Thrombopoietin (TPO), the ligand for the c-Mpl receptor, is a major regulator of megakaryopoiesis [8,9]. It stimulates the formation and proliferation of megakaryocyte colonies from human $CD34^+$ cells [10] as well as

*Correspondence to: Motoki Bonno, Department of Pediatrics, Mie University School of Medicine, 2-174, Edobashi, Tsu-city, Mie 514, Japan. E-mail: shouni@clin.medic.mie-u.ac.jp

Received for publication 16 October 1997; Accepted 8 April 1998

TABLE I. Characteristics of Patients*

Patient	GA (week)/ BBW (g)	Onset (day)/ sex	Surface marker	PPO	Complication	Karyotype	Prognosis	Platelets ($\times 10^9$/l)	Leukocytes ($\times 10^9$/l)	Blast (%)	TPO level (fmol/ml)
1	33/2,882	0/M	CD7, 33, 34, 41	+	jaundice VSD, PDA	47 XY +21	CR	107	95.4	84	0.36
2	37/1,912	4/M	CD7, 33, 34, 41	ND	hepatic failure ECD	47 XY +21	Dead (day 45)	106	45.9	48	0.93
3	34/2,858	0/F	CD7, 33, 34, 41	+	hepatic failure PDA	47 XY +21	Dead (day 43)	493	158.7	86	<0.20
4	36/2,322	5/M	CD33, 34, 41$^{\pm}$	+	PDA	46 XY −21, +t(21q, 21q)	Alive, relapsed with M7	143	66.2	22	0.55
5	37/2,404	15/M	CD7, 33, 34, 41	ND	VSD	47 XY +21, inv (+11q13)	CR	1040	36.1	23	0.30
6	37/3,150	52/F	CD7, 33, 34, 41	+	VSD, PDA, ASD	47 XY +21	CR	42	12.4	45	0.46

*PPO, platelet peroxidase; VSD, ventricular septal defect; PDA, patent ductus arteriosus; ECD, endocardial cushion defect; CR, complete remission; ND, not determined.

maturation of megakaryocytes, resulting in increased megakaryocyte size and ploidy with profound increases in platelet count [9]. Several studies on blood TPO levels in various hemopoietic disorders have revealed that the levels are variable and may correlate with megakaryocyte mass rather than platelet count [11–13].

Because increased megakaryoblastic cells spontaneously regress in TMD, it is of special interest to consider the relationship between the blood TPO levels and the blast numbers. In this study, we measured the blood TPO levels serially using an ELISA assay in six patients with TMD. Furthermore, c-Mpl expression on the blast cells was examined.

MATERIALS AND METHODS

Subjects

Blood TPO levels were measured in six patients with TMD from April 1994 to April 1997. The diagnoses of TMD were made according to a previous report [4]: (1) Down syndrome confirmed by chromosomal analysis, (2) abnormal peripheral blood picture indistinguishable from acute leukemia, which is normalized or improved during the natural course without chemotherapy; (3) the patient is neonate. Patient details are summarized in Table I. There were four males and two females, median gestational age was 36.5 weeks (range 33–37), and birth body weight was 2,588 ± 455.4 g. In all patients, blast cells were megakaryocytic lineage, which was confirmed by expression of CD41 and/or positive stain for platelet peroxidase (PPO). Chromosomal analyses of blast cells showed 21 trisomy in five patients and translocation Down syndrome in one patient. Additional chromosomal abnormality (chromosome inversion) was found in one patient. All patients had liver dysfunction to some extent during the course: three patients had severe jaundice, and two of three died of hepatic failure. One patient developed acute megakaryoblastic leukemia at 1 year of age. As a control, six neonates were selected. They were admitted in a neonatal care unit due to nonhematological disorders such as eosinophilic gastroenteritis, hydrocephalus, congenital lung cyst, anal atresia, low body weight, and pneumothorax (three males and three females; median gestational age 39 weeks, range 32–41; birth body weight 2,746 ± 841.2 g).

Measurement of Thrombopoietin (TPO)

Serial blood samples were collected from patients. Serum was separated and stored at −20°C until assayed. Plasma anticoagulated with citrate was used for two samples. TPO was measured using ELISA kit (Kirin Brewery Co., Tokyo, Japan) as previously described [11]. The lowest detectable level of TPO was 0.20 fmol/ml (0.028 mol/ml TPO represents 1 pg/ml TPO).

Immunofluorescence Analysis of c-Mpl on the Blast Cells

For analyses of c-Mpl expression, mononuclear cells were separated from patients and stored at −70°C until use. c-Mpl expression on blast cells was determined by two-color flow cytometry using rabbit anti-human c-Mpl polyclonal antibody ([14]; Kirin Brewery Co.) and FITC-conjugated mouse anti-CD41 and/or -CD34 monoclonal antibodies (Immunotech, Hamburg, Germany; Becton Dickinson, Mountain View, CA, respectively). Indirect immunofluorescence staining for c-Mpl expression was performed using phycoerythrin-conjugated goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, MO). As a control, preimmune rabbit IgG (Sigma Chemical Co.) was applied. c-Mpl expression was analysed by using FACScan (Becton Dickinson). Cell line CMK11-5 was used as a positive control for c-Mpl expression [15].

Statistical Analyses

The Student's *t*-test was used for the calculation of differences between groups. Pearson correlation coefficient was used for the calculation of correlations between TPO and other hematological data. Values for TPO levels, blast numbers, and platelet counts were logarithmically transformed because those values were not normally distributed.

RESULTS

TPO Levels in TMD at Onset

Clinical data and TPO levels of TMD patients at the onset are shown in Table I. At the onset of the disease, megakaryoblastic cells were observed in the peripheral blood at various degrees (mean 47.5%, range 22–86). TPO was under detectable level in one patient who had the highest number of blast cells (Patient 3). Five patients had a mean TPO level of 0.52 fmol/ml (0.30–0.93). TPO levels in TMD patients were significantly lower than in controls (mean 3.70 fmol/ml, range 1.38 to 8.33, $P < 0.001$). Platelet counts were various in TMD patients at onset: one patient (Patient 5) was thrombocytemic and another (Patient 6) was thrombocytopenic. The mean platelet count of TMD patients (321×10^9/l, range 42–1,040) was similar to that of controls (253×10^9/l, range 124–381).

Clinical Course and TPO Levels of Patients With TMD

Blast cells in the peripheral blood were spontaneously regressed in all patients with TMD within several weeks. Five patients developed thrombocytopenia during the course and three of them required transfusion of platelet, packed red blood cells, and fresh frozen plasma because of hemorrhagic diathesis due to disseminated intravascular coagulation (Patients 1, 2, and 3). Patient 1 received platelet transfusion on day 7 and 9 (platelet count: 44 and 31×10^9/l), Patient 2 on day 7 and 8 (49 and 38×10^9/l), and Patient 3 on day 14, 17, 25, and 26 (213, 105, 64, and 105×10^9/l). The response to platelet transfusion was poor without fresh frozen plasma. After regression of the blast cells, platelet counts were increased to the normal range in two patients (Patient 1 and 4), while platelet counts remained less than 100×10^9/l in three patients (Patient 2, 3, and 6, Fig. 1C). TPO levels were measured in five patients after spontaneous regression of the blast cells. With regression of blast cells, TPO levels increased ten- to twenty-fold in four survived patients and reached to the similar levels as controls. In one patient with hepatic failure, TPO level was poorly elevated and relatively lower compared to the others (Patient 2).

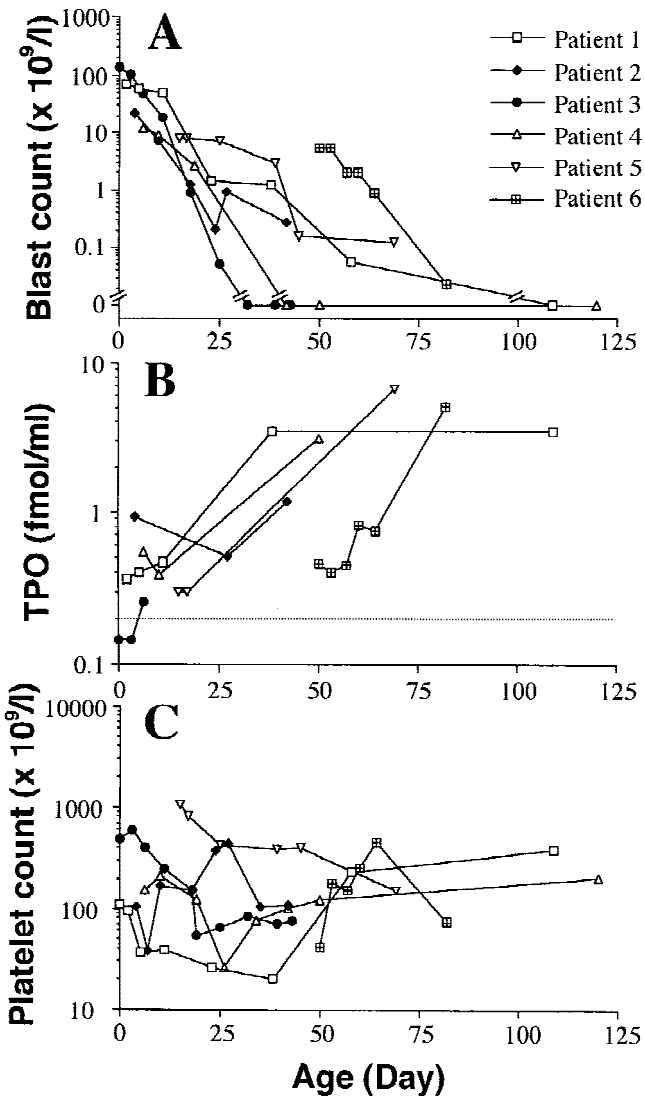

Fig. 1. Blast count, serum TPO level, and platelet count in six TMD patients during the course. A: Blast counts were spontaneously decreased in all TMD patients. B: Blood TPO levels were increased while blast counts were decreased. C: Platelet counts were altered during the course.

Reciprocal Relationship Between Serum TPO Levels and Blast Numbers

Figure 2 shows the relationship of blast numbers in the pheripheral blood and TPO levels. There was a significant correlation between TPO levels and blast numbers ($r = -0.85$, $P < 0.001$). There was no significant correlation between TPO levels and platelet counts ($r = 0.426$).

c-Mpl Expression on Blast Cells

c-Mpl expression was examined on blast cells from four TMD patients. All patients had positive stain for c-Mpl on the blast cells (Fig. 3).

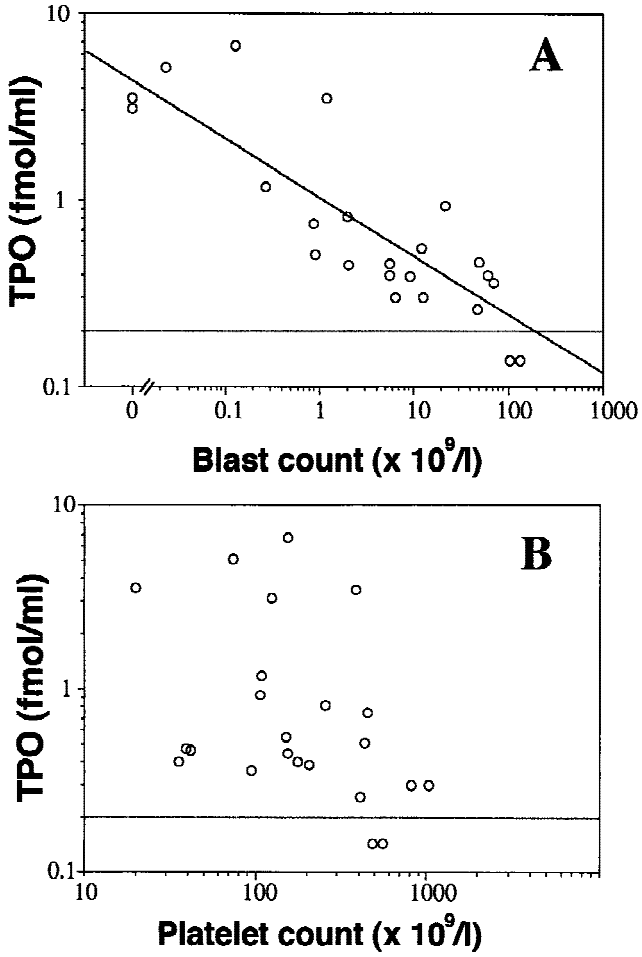

Fig. 2. A: Relationship with blood TPO level and blast count in six TMD patients. There was a significant correlation between the two parameters ($r = -0.85$, $P < 0.001$). B: Relationship between blood TPO level and platelet count in TMD patients. There was no significant correlation ($r = 0.426$).

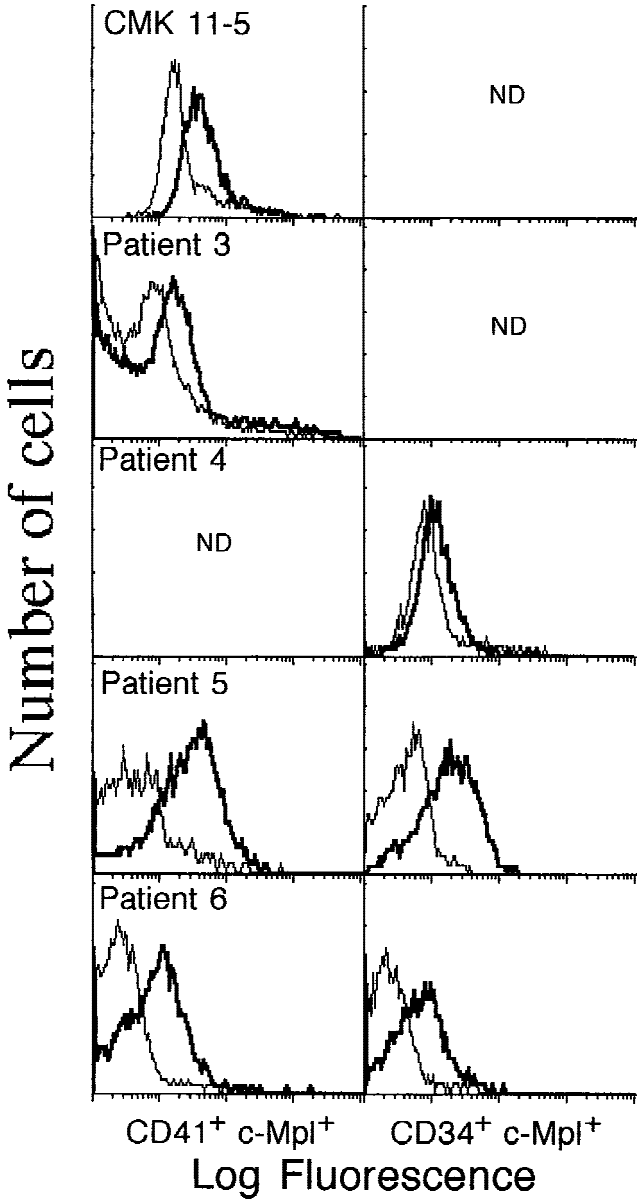

Fig. 3. c-Mpl expression on blast cells. Blast cells were stained for CD41 and/or CD34 in combination with c-Mpl. Cell line CMK 11-5 was used as a positive control for c-Mpl expression. CMK 11-5 and blast cells from four TMD patients were all positive for c-Mpl expression. ND: not determined.

DISCUSSION

In this study, we have shown that TPO levels were considerably decreased in patients with TMD while plentiful megakaryoblastic cells were circulating in the peripheral blood. A mean TPO level in TMD at the onset was one-seventh of a mean level in neonates who had equivalent platelet counts. TPO levels were not correlated with platelet counts; however, elevation of TPO levels was correlated inversely with reduction of blast cell mass bearing c-Mpl, which is a receptor for TPO.

Recently, serum TPO levels in healthy Japanese children have been reported [16]. According to the report, mean TPO levels were highest at the second day of age (5.92 ± 1.42 fmol/ml), high before 2 months of age (3.73–4.32 fmol/ml), and stable from 2 months to 4 years (1.97–2.23 fmol/ml). Even compared with these data, mean TPO level at the onset in TMD was considerably low.

It has been reported that TPO levels may correlate with megakaryocyte mass rather than platelet counts [12,13], and postulated that TPO levels are regulated by TPO uptake and catabolism through c-Mpl receptor on platelets and megakaryocytes [17]. Although TMD is not a normal physiological condition, inverse correlation between TPO levels and the numbers of blast cells is consistent with a theory that megakaryocyte mass is a major regulator of blood TPO levels, and low TPO levels in TMD patients at onset might be caused by absorbing

TPO through c-Mpl on blast cells and elevation of TPO levels after spontaneous regression of blast cells might be due to the decrease of c-Mpl-bearing cells.

Platelet counts were elevated in spite of low TPO levels in two TMD patients (Patients 3 and 5). It has been reported that certain acute myeloblastic leukemia cells coexpressed both TPO and c-Mpl genes. We examined the conditioned medium from culture of the blast cells, and a low concentration of TPO with a considerable amount of IL-6 was detected from one TMD patient (Bonno et al., 1997, unpublished observation). It is speculated that TPO may be synthesized by the blast cells and utilized in an autocrine manner to stimulate differentiation of the blast cells and platelet production, synergistically with other cytokines, such as IL-6.

In two patients, severe liver dysfunction was already obvious at the onset and hepatic failure rapidly progressed after regression of the blast cells (Patients 2 and 3). In these two patients, diffuse intralobular liver fibrosis with massive destruction of the hepatic architecture was observed at necropsy. In Patient 2, TPO level was poorly elevated and relatively lower after regression of blast cells compared to the others without hepatic failure. Because liver is a major source of TPO production [18], low TPO levels might be due to failure of TPO production in the liver.

Pathogenesis of TMD is unknown. Trisomy 21 seems to play an important role in abnormal proliferation of megakaryoblastic cells. However, either TPO or c-Mpl gene is not located on chromosome 21; TPO gene is located on chromosome 3q27 [19] and c-Mpl on 1p34 [20]. There might be unknown abnormalities on other regulatory factors for megakaryopoiesis or signal transduction of c-Mpl, which might be located on chromosome 21.

It has been reported that TPO has a proliferative effect on certain acute myeloblastic leukemia cells [21]. The role of TPO on megakaryopoiesis in TMD is unclear. However, our findings may provide further insights into TPO regulation.

ACKNOWLEDGMENTS

We thank Drs. Yoshio Amemiya, Hiroshi Taneda, Hiroshi Sawada, and Hidehito Goto (Mie Prefectural General Hospital and Mie University School of Medicine, Mie, Japan) for providing blood samples. Special thanks go to Masato Hosoda (Pharmaceutical Research Laboratory, Kirin Brewery Co. Ltd., Gunma, Japan) for helpful and valuable discussion and suggestions throughout this work.

REFERENCES

1. Elizabeth A, Cairney L, Mckenna R, Arthur DC, Nesbit ME, Wood WG: Acute megakaryoblastic leukemia in children. Br J Haematol 63:541–555, 1986.
2. Kojima S, Matsuyama T, Sato T, Horibe K, Konishi S, Tsuchida M, Hayashi Y, Kigasawa S, Akiyama Y, Okamura J, Nakahata T, Bessho F, Eguchi M, Nakazawa S, Ueda R: Down's syndrome and acute leukemia in children: Analysis of phenotype by use of monoclonal antibodies and electron microscopic platelet peroxidase reaction. Blood 76:2348, 1990.
3. Engel RR, Hammond D, Eitzman DV, Pearson H, Krivit W: Transient congenital leukemia in 7 infants with mongolism. J Pediatr 65:303–305, 1964.
4. Miyauchi J, Ito Y, Kawano T, Tsunematsu Y, Shimizu K: Unusual diffuse liver fibrosis accompanying transient myeloproliferative disorder in Down's syndrome: A report of four autopsy cases and proposal of a hypothesis. Blood 80:1521–1527, 1992.
5. Brodeur GM, Dahl GV, Williams DL, Tipton RE, Kalwinsky DK: Transient leukemoid reaction and trisomy 21 mosaicism in a phenotypically normal newborn. Blood 55:691–693, 1980.
6. Heaton DC, Fitzgerald PT, Fraser GJ, Abbott GD: Transient leukemoid proliferation of cytogenetically umbalanced +21 cell line of a constitutional mosaic boy. Blood 57:883–887, 1981.
7. Rowley JD: Down syndrome and acute leukemia: Increased risk may be due to trisomy 21. Lancet 2:1020, 1981.
8. Kato T, Ogami K, Shimada Y, Iwamatsu A, Sohmaa Y, Akahori H, Horie K, Kokubo A, Kudo Y, Maeda E, Kobayashi K, Ohashi H, Ozawa T, Inoue H, Kawamura K, Miyazaki H: Purification and characterization of thrombopoietin. J Biochem 118:229–236, 1995.
9. Kaushansky K, Broudy VC, Lin N, Jorgensen MJ, McCarty J, Fox N, Zucker-Franklin D, Lofton-Day C: Thrombopoietin, the Mpl ligand, is essential for full megakaryocyte development. Proc Natl Acad Sci USA 92:3234–3238, 1995.
10. Nichol JL, Hokom MM, Hornkohl A, Sheridan WP, Ohashi H, Kato T, Li YS, Bartley TD, Choi E, Bogenberger J, Skrine JD, Knudlen A, Chen J, Trait G, Sleeman L, Cole S, Grampp G, Hunt P: Megakaryocyte growth and development factor: Analysis of in vitro effect on human megakaryopoiesis and endogenous serum levels during chemotherapy-induced thrombocytopenia. J Clin Invest 95:2973–2978, 1995.
11. Tahara T, Usuki K, Sato H, Ohashi H, Morita H, Tsumura H, Matsumoto A, Miyazaki H, Urabe A, Kato T: A sensitive sandwich ELISA for measuring thrombopoietin in human serum: Serum thrombopoietin levels in healthy volunteers and in patients with haemopoietic disorders. Br J Haematol 93:783–788, 1996.
12. Marsh JCW, Gibson FM, Prue RL, Bowen A, Dunn VT, Hornkohl AC, Nichol JL, Gordon-Smith EC: Serum thrombopoietin levels in patients with aplastic anaemia. Br J Haematol 95:605–610, 1996.
13. Emmons RVB, Reid DM, Cohen RL, Meng G, Young NS, Dunbar CE, Shulman NR: Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. Blood 87:4068–4071, 1996.
14. Morita H, Tahara T, Matsumoto A, Kato T, Miyazaki H, Ohashi H: Functional analysis of the cytoplasmic domain of the human Mpl receptor for tyrosine-phosphorylation of the signaling molecules, proliferation and differentiation. Fed Eur Biochem Soc Lett 395:228–234, 1996.
15. Sasaki A, Katoh O, Kawaishi K, Hyodo H, Kimura A, Satow Y, Kuramoto A: Expression of c-Mpl and c-Mpl ligand gene in hematopoietic cells of individuals with and without myeloproliferative disorders and leukemia cell lines. Int J Hematol 62:217–223, 1995.
16. Ishiguro A, Nakahata T, Matsubara K, Hayashi Y, Tahara T, Kato T, Shimbo T: Age-related changes in serum thrombopoietin levels in children. Exp Hematol 25:900, 1997.

17. Kuter DJ, Rosenberg RD: The reciprocal relationship of thrombopoietin (c-mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. Blood 85:2720, 1995.
18. Sungaran R, Markovic B, Chong BH: Localization and regulation of thrombopoietin mRNA expression in human kidney, liver, bone marrow, and spleen using in situ hybridization. Blood 89:101–107, 1997.
19. Sohma Y, Akahori H, Seki N, Hori T, Ogami K, Kato T, Shimada Y, Kawamura K, Miyazaki H: Molecular cloning and chromosomal localization of the human thrombopoietin gene. Fed Eur Biochem Soc Lett 353:57–61, 1994.
20. Le Coniat M, Souyri M, Vigon I, Wendling F, Tambourin P, Berger R: The human homolog of the myeloproliferative virus maps to chromosome band 1p34. Hum Genet 83:194–196, 1989.
21. Quentmeier H, Zaborski M, Graf G, Ludwig WD, Drexler HG: Expression of the receptor MPL and proliferative effects of its ligand thrombopoietin on human leukemia cells. Leukemia 10:297–310, 1996.